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ORIGINAL ARTICLE

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Atypical features of rat dentate granule cells: recurrent basal dendrites and apical axons

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Abstract The stereotyped morphology of dentate granule cells in rodents consists of apical dendrites arborizing in the molecular layer and an axon arising from the opposite pole of the soma. Recently, we showed that epilepsy induces the formation of basal dendrites on granule cells and that these dendrites extend into the hilus of the dentate gyrus. The present Golgi study of granule cells from adult rats shows two atypical features for granule cells in control rats. One is the occurrence of recurrent basal dendrites (RBDs) that are defined as basal dendrites arising at or near the hilar pole of the soma and then curving back to the molecular layer. The frequency of granule cells with RBDs was 3.8% in control rats. The second is apical axons of granule cells that were observed to originate from either the apical pole of the soma or an apical dendrite. The incidence of these "apical" axons was about 1%. These morphological findings in the present study suggest that rat granule cells are more heterogeneous than previously indicated. Furthermore, their frequency was not increased in epileptic rats.

Keywords Golgi method · hilus · basal dendrites · apical dendrites · mossy fibers

Introduction

The classical studies in rats and mice indicated that hippocampal dentate granule cells are bipolar cells with apical dendrites arising from one pole and an axon from the other (Lorente de Nó 1934; Blackstad 1963; Laatsch and Cowan 1966; Lindsay and Scheibel 1981; Seress and Pokorny 1981; Desmond and Levy 1982). This morphology is assumed to be the same for all granule cells in rats (reviewed Freund and Buzsáki 1996). Recently, spiny and branched dendrites in the subgranular region of the hilus were shown to arise from the base of granule cells in epileptic rats following perforant-path stimulation (Spigelman et al. 1998). In particular, the basal dendrites that distributed in the hilus, i.e., the "hilar basal dendrites" (HBDs), were seen on nearly 10% of the granule cells in the epileptic rats. However, none were observed in the control rats (Spigelman et al. 1998).

During the course of this latter study of HBDs, we noticed in the same Golgi material that some dendrites arose from the basal half, or basal pole, of the soma but quickly curved toward the molecular layer. These dendrites are different from HBDs because they curve back to the molecular layer where they join the distribution field of the apical dendrites. For this reason, such dendrites are defined as "recurrent basal dendrites" (RBDs) in the present study. Although the morphology of dentate granule cells is well documented in previous studies, descriptions of the dendritic arbors of granule cells failed to show RBDs in normal rats. However, one previous report indicated the presence of granule cells with RBDs in the human dentate gyrus (Seress and Mrzljak 1987). The present study was undertaken to gain information about the morphology, sublaminar location and frequency of dentate granule cells with RBDs in normal adult rats using Golgi preparations. In addition, apical axons, another morphological feature that was not previously described for rat granule cells, are reported in the current investigation. Effort was also taken to determine whether any change occurs in the frequency of either of these two atypical features of granule cells in perforant path stimulated epileptic rats.

Materials and methods

The methodology for perforant path stimulation with implanted electrodes was described in detail by Spigelman et al. (1998), and the Golgi material analyzed in the present study was identical to

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that in the above paper. Animal protocols were pre-approved by the Institutional Animal Care and Use Committee at the University of California. Golgi preparations were made from the brains of experimental rats ($n = 4$) and age-matched controls ($n = 4$). Briefly, animals were perfused intracardially with cold saline followed by 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer. The caudal cerebrum containing the hippocampal formation was blocked into 3-mm-thick slices and processed with a modified rapid Golgi method (Spigelman et al. 1998). Blocks from experimental and control brains were immersed in osmium tetroxide (0.25%) and potassium dichromate (9%) for 5 days in the dark. The blocks were then rinsed several times with 0.75% silver nitrate and placed in this latter solution for 2 days in darkness. Blocks were sectioned at 70 µm, and the sections were dehydrated in a graded series of ethanols followed by xylene. Finally, sections were placed on glass slides, and coverslips applied with a mounting medium.

Granule cells in the hippocampal dentate gyrus were readily identified by the size, shape and location of the soma, and were examined for RBDs. Also analyzed was the apical axon, an axon arising from either the upper part of the soma or the apical dendrite. For the quantitative analysis, the granule cell layer of both hemispheres was scanned and imaged over the entire length and depth with ×40 and ×100 objectives. Particular attention was paid to the origins of primary dendrites and axons. The criteria for distinguishing between dendritic and axonal processes included their thickness, branching pattern and the presence or absence of spines. Thus, dendrites, either apical or basal, had a thicker diameter and always had spines while axons were thin along their entire course except for the large mossy fibers. Only well-impregnated granule cells that showed dendritic and axonal processes were counted in this analysis. The location within the granule cell layer was noted for the soma of granule cells with RBDs and apical axons. Images of granule cells were obtained with a digital video camera and a light microscope, and montages of impregnated granule cells at different focal planes were made using Adobe Photoshop software. The percentage of cells with atypical dendrites or axons was calculated in both control and epileptic rats. The Student's *t*-test was used to determine any significant differences.

Results

Typical granule cells

Golgi preparations from control rats show granule cells with classical features (Lorente de Nó 1934; Blackstad 1963). These cells have round or oval somata (Fig. 1A). Most of the oval somata located in the lower part of the granule cell layer are oriented with their long axis perpendicular to the plane formed by the hilus and this layer. The somata of many granule cells taper at their base and their axons arise from this location where it is directed toward the hilus (Fig. 1A). A variable number of dendrites arises from the apical pole, and they branch at varying distances from the soma before extending into the molecular layer and terminating at the hippocampal fissure. These dendrites are distinguishable from the axon by a greater thickness and spines on the dendritic shaft.

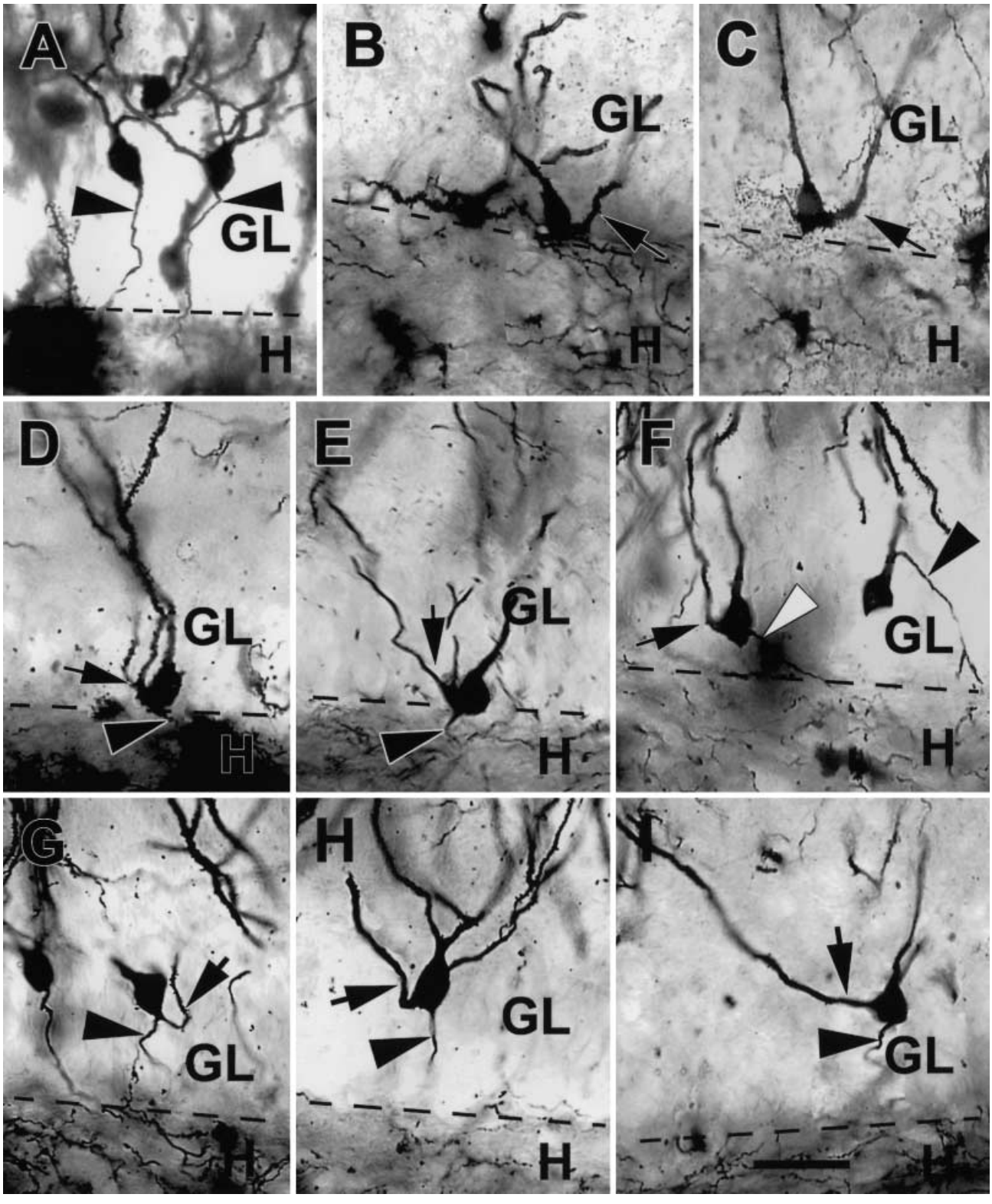
Granule cells with RBDs

Golgi preparations from control rats also show granule cells with RBDs, a feature that was not included in the classical description of these cells or in the previous description of hilar basal dendrites present in epileptic rats (Spigelman et al. 1998). RBDs are defined as basal dendrites that arise from the lower portion of the granule cell body, either at or near the hilar pole, and then curve back toward the molecular layer. The site of emergence of these dendrites is similar to that for basal dendrites on neocortical pyramidal neurons (Feldman 1984). As shown in Fig. 1 B–E, granule cells with their somata within the lowest part of the layer bordering the hilus may have such a dendritic process that arises from the basal portion of their somata. These dendrites are spinous and usually have the same thickness (0.5–1.5 µm) as the apical dendrite but in rare instances they can be thicker (2.5 µm) at their origin (Fig. 1B). Within a distance of about 10 µm, this process changes direction and penetrates the granule cell layer to reach the molecular layer. Some of the RBDs extend to the hippocampal fissure. Most of the granule cells with RBDs are found in this position, adjacent to the hilar border.

Granule cells with RBDs are not limited to the hilar border of the granule cell layer because some are found in the middle of the granule cell layer (Fig. 1F–I). The RBDs from these granule cells were seen to arise from either the lower half of the soma (Fig. 1F, H, I) or the hilar pole (Fig. 1G). Note that these granule cells have their axons arise from the base of the soma (Fig. 1H, I) or from the origin of the RBD (Fig. 1G). Granule cells with RBDs were found in both the upper and lower blades of the granule cell layer, without any particular topographic preference across the granule cell layer.

The location and frequency of granule cells with RBDs were determined in both control and epileptic rats. Granule cells with RBDs were mainly located within one cell-diameter distance from, or at, the hilar border (73% in control and 77% in epileptic rats). The remaining granule cells with RBDs resided in the middle of the granule cell layer. There were 33 examples of RBDs from 866 granule cells obtained from control rats (3.8%). A similar percentage was obtained from epileptic rats (31 from 861; 3.6%). No statistical difference was found between the two groups in regard to the frequency or sublaminar location of granule cells with RBDs.

Fig. 1A–I Photomicrographs of Golgi-impregnated dentate granule cells. **A** Two granule cells in the middle of the granule cell layer (*GL*) that display typical features such as apical dendrites directed into the molecular layer and an axon (*arrowheads*) from the opposite pole entering the hilus (*H*). **B–I** Examples of granule cells with RBDs. RBDs (*arrows*) originate from the hilar pole or lower half of granule cell bodies and then they curve upwards to enter the molecular layer. **B–E** Granule cells with RBDs that have their somata located at the border (*broken lines*) between the GL and H. RBDs of these cells were frequently connected to the hilar pole of the soma next to the site of origin of the axon (*arrowheads*). **F–H** show granule cells with RBDs that have somata in the middle of the granule cell layer. Occasionally, as in **G**, the RBD (*arrow*) and axon (*arrowhead*) arose from the same point on the soma. Axons of granule cells with RBDs most often arose from the hilar pole (**E,H,I**), but sometimes had their origin from the side of the soma (*white arrowhead* in **F**). **F** Also shows a granule cell with an axon (*black arrowhead*) arising from an apical dendrite. **A,E–I** were from control rats while panels **B–D** were from epileptic rats. **B**ar 20 µm



Granule cells with axons arising from apical dendrites

In both control and epileptic preparations, some granule cells without an axon at their base were found. In these cases, the axon was observed to arise from two different locations. Thus, granule cell axons arose from the apical dendrite about 10 µm from the soma (Fig. 1F, 2E) or at the apical dendrite's origin (Fig. 2A, C, D). Granule cells with their long axis oriented parallel to the plane of the granule cell layer and hilus often had their axons arise from one of the two dendrites (Fig. 2B, F) instead of from its basal surface, the side of the cell body closest to

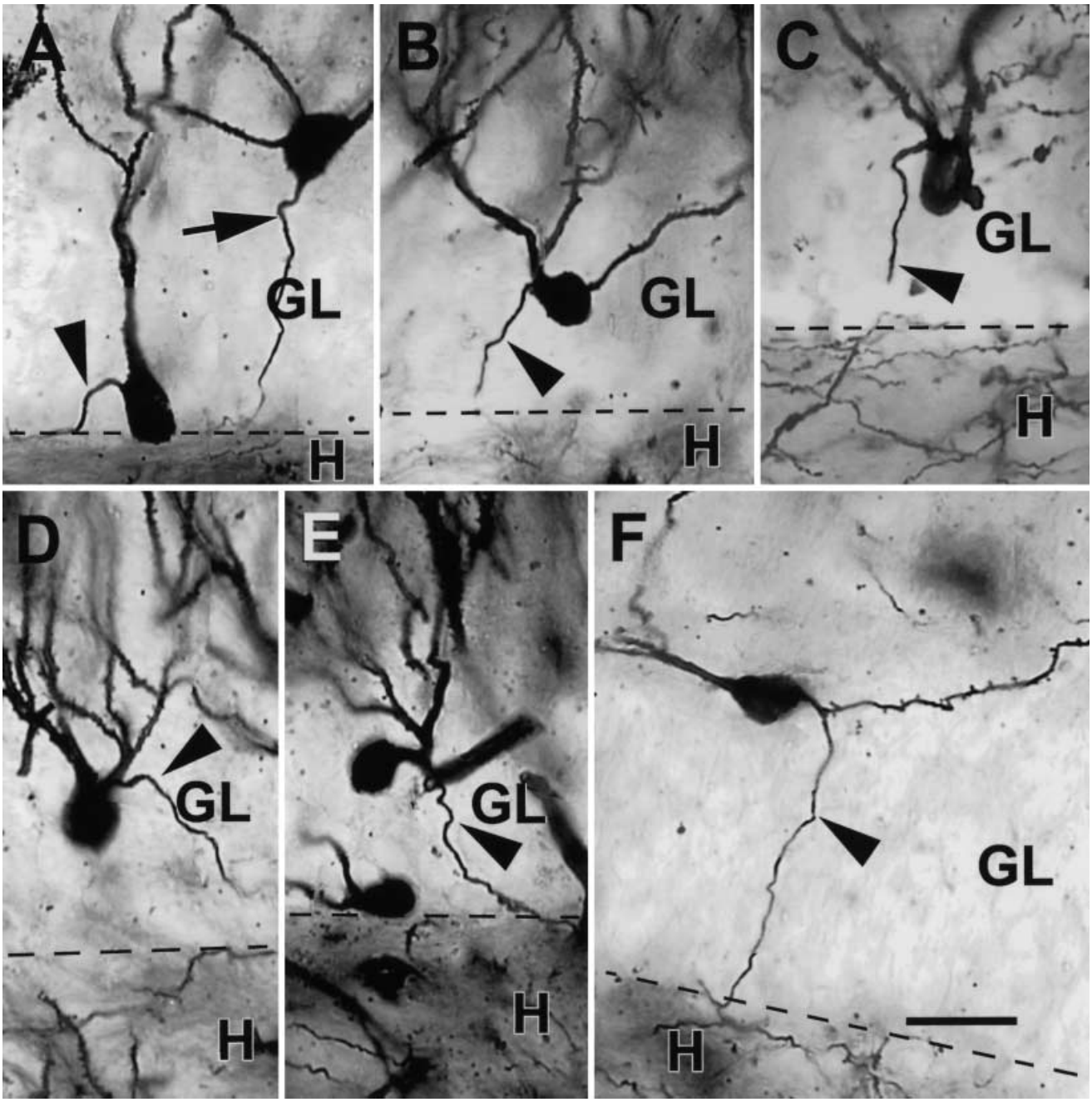


Fig. 2A–F Photomicrographs of dentate granule cells with apical axons or axons arising from a proximal dendrite. **A–C** Granule cells with axons (*arrowheads*) that arise from the upper half of the soma close to the point where the apical dendrite originates. The other impregnated granule cell in **A** has its axon (*arrow*) arise from the basal pole. **D, E** Axons (*arrowheads*) arising directly from apical dendrites at varying distances from their parent somata (also see **Fig. 1**, the granule cell on the right side of **F**). **F** shows a granule cell located at the border between the granule cell and molecular layers. It has its long axis oriented parallel to this border. Note that its axon (*arrowhead*) arises from one of the two dendrites that emerge from opposite poles of this cell body. *Broken lines* indicate the border between the granule cell layer (*GL*) and hilus (*H*). **B,D** from control rats; **A,C,E,F** from epileptic rats. Bar 20 µm

the hilus. In all cases, these axons were directed toward the hilus. Some of the apical axons could be followed into the hilus and collaterals were observed. These "apical" axons were found on granule cells located at different levels of the granule cell layer showing no preference for the lower part of this layer as did those with RBDs. There were 10 examples of apical axons from 866 granule cells obtained from control rats (1.2%). Epileptic rats showed a similar percentage for the frequency of granule cells with apical axons (8 from 861, 0.9%).

As previously shown, granule cells with HBDs are commonly found in epileptic brains (Spigelman et al. 1998), and their axons were previously shown to arise from the base or side of their somata. During the course

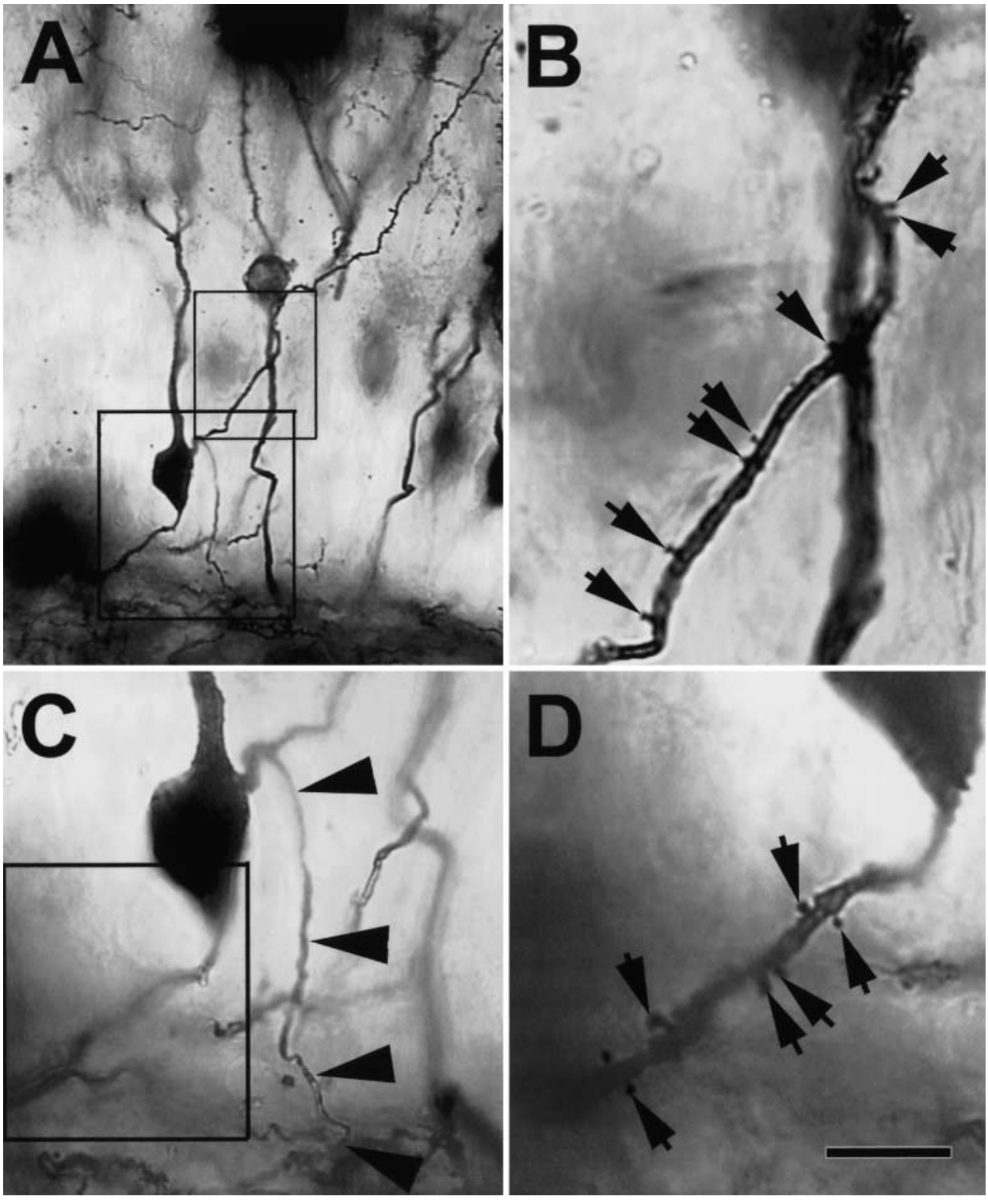


Fig. 3A–D Photomicrographs of a dentate granule cell from an epileptic brain. This cell has a hilar basal dendrite arising from its basal pole and an axon arising from the proximal portion of an apical dendrite. **A** Overall view of this cell and its position in the lower part of the granule cell layer. Note that one of the apical dendrites is thick and branched while the second apical dendrite is thin. The upper box in **A** is enlarged in **B** to show the spines (*arrows*) on the thin apical dendrite. **C** Enlargement of the lower box in **A** to show the origin of this looping process, an axon (*arrowheads*), from the base of the thin dendrite. The axon ran into the hilus, tapered down to a thin process and lacked spines. **D** shows the hilar basal dendrite (box in **C**) at a different focal plane to demonstrate its spines (*arrows*). Bar 20 µm for **A**; 10 µm for **C;** 5 µm for **B,D**.

of the present study, we noticed that a few granule cells with HBDs had their axons arising from an apical dendrite. These examples were not reported in the previous paper by Spigelman et al. (1998). One of these cells is shown in Fig. 3. Note that the soma of this granule cell is located close to the hilar border (Fig. 3A, C), and a spiny dendrite, a HBD, arises from its base and enters the hilus (Fig. 3D). The apical axon (Fig. 3C) arises from one of its two apical dendrites (Fig. 3B). This example indicates that granule cells with HBDs may also have "apical" axons.

Discussion

The major findings of the present study are the light microscopic descriptions of "recurrent basal dendrites (RBDs)" and "apical axons" for adult rat dentate granule cells. These two morphological observations represent exceptions to the stereotyped features of rat granule cells that include: (1) an oval or round soma with its long axis lying perpendicular to the granule cell layer; (2) dendrites that are all assumed to originate from the somal pole facing the molecular layer and arborize exclusively in the molecular layer; (3) an axon arising from the hilar pole of the soma and invading the hilus (Lindsay and Scheibel 1981; Seress and Pokorny 1981; Desmond and Levy 1982, Claiborne et al. 1990; Rihn and Claiborne 1990; Scharfman et al. 1990). Some of these studies utilized Golgi preparations that show the dendrites and somata of selectively impregnated neurons (Lindsay and Scheibel 1981; Seress and Pokorny 1981; Desmond and Levy 1982). Other studies utilized individually injected granule cells (Claiborne et al. 1990; Rihn and Claiborne 1990; Scharfman et al. 1990). One of these latter studies only sampled granule cells in the outer two-thirds of the granule cell layer (Rihn and Claiborne 1990). Thus, granule cells in the inner portion of this layer, including those at the hilar border, were probably not examined. This point is pertinent because most (about 75%) of the granule cells with RBDs are located near the hilar border of the granule cell layer. In addition, the frequency of these two atypical features for granule cells is low in the dentate gyrus of adult control rats. Thus, only about 4% of granule cells have RBDs and 1% of granule cells possess apical axons.

The length and thickness of RBDs were shown to vary in this analysis. Some RBDs were thicker at their origin than others. Similarly, some of the RBDs were followed into the molecular layer while some appeared to terminate in the granule cell layer. Thus, the arborization of RBDs was not as extensive as that of the apical dendrites in the molecular layer. RBDs are often not branched within the entire granule cell layer, and even in the molecular layer they only divide into one or two branches. Similar to apical dendrites, RBDs have spines that are located along their entire length. On the other hand, RBDs arise from the basal pole of the soma, and thus, are closer to the origin of the axon than apical dendrites. The similarity and differences between RBDs and apical dendrites stress the importance for further investigations on the synaptic input of RBDs.

As mentioned above, granule cells with RBDs are mostly localized to the lower portion of the granule cell layer. Recently, studies have shown that granule cells are constantly generated in normal adult rats and the rate of neurogenesis increases following a variety of physiological and pathological stimuli, including increased environmental exploration, increased exercise, and status epilepticus (Bengzon et al. 1997; Parent et al. 1997; Gray and Sundstrom 1998; Gould et al. 1999; van Praag et al. 1999). The newly generated granule cells arise from stem cells that reside in the subgranular zone of the hilus, and then they migrate toward the molecular layer side of the granule cell layer (Parent et al. 1997). Thus, the inner portion of the granule cell layer contains the newly-generated granule cells implying that granule cells with RBDs may represent a comparatively younger population of the granule cell pool.

The Golgi preparations used in this analysis showed that RBDs and apical axons were also observed for granule cells from epileptic rats. The frequency of both of these atypical features was not significantly different from that for the control rats. Thus, RBDs are unlike HBDs because granule cells with HBDs were commonly observed in epileptic rat brains and were rarely found in control rats (Spigelman et al. 1998; Buckmaster and Dudek 1999; Ribak et al. 2000). Also, HBDs were shown to be postsynaptic to mossy fibers, and thus they provide an additional basis for increased excitatory circuitry in epileptic rats (Ribak et al. 2000). This difference between the two types of dendrites arising from the base of granule cells is of great interest because the origin of both dendrites is the same location on the soma. It may be expected that mechanisms exist for granule cell dendrites to be distributed only in the molecular layer in normal brains even though they emerge from the hilar pole or lower half of the soma. Some of these mechanisms may include trophic factors in the molecular layer and/or repelling factors in the hilus (Bender et al. 1998).

Although the present study is the first to describe apical axons on granule cells of mammalian species, we are hesitant to draw any particular biological significance or function for this structure. Earlier studies mentioned that apical axons are present on dentate interneurons such as basket cells (see Ribak and Seress 1983), but these neurons are considered to be much more heterogeneous in morphology than dentate granule cells. Granule cells with HBDs also showed apical axons. Although these latter granule cells had their somata by the hilar border, other granule cells with apical axons had somata closer to the molecular layer. In all of these cases, these axons were directed toward the hilus and appeared to travel in the same direction (into the hilus and toward CA3) with axons arising from the base of granule cells.

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